--- ----- -----------
? s ribozym? and trans? and intron?

>>>File 155 processing for TRANS? stopped at TRANSESOPHAGIAL
>>>File 5 processing for TRANS? stopped at TRANSFECTABLE

	4090	RIBOZYM?
	805623	TRANS?
	39783	INTRON?
S1	166	RIBOZYM? AND TRANS? AND INTRON?

? rd

...examined 50 records (50)
...examined 50 records (100)
...examined 50 records (150)
...completed examining records

S2	112	RD (unique items)

? s s2 and mutant?

	112	S2
	288268	MUTANT?
S3	21	S2 AND MUTANT?

? t s3/3,ab/all

3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09972990 99177329
Trans-activation of the Tetrahymena group I **intron** **ribozyme** via a non-native RNA-RNA interaction.
Ikawa Y; Shiraishi H; Inoue T
Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606-8502, Japan.
Nucleic Acids Res (ENGLAND) Apr 1 1999, 27 (7) p1650-5, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The peripheral P2.1 domain of the Tetrahymena group I **intron** **ribozyme** has been shown to be non-essential for splicing. We found, however, that separately prepared P2.1 RNA efficiently accelerates the 3' splice-site-specific hydrolysis reaction of a **mutant** **ribozyme** lacking both P2.1 and its upstream region in **trans**. We report here the unusual properties of this **trans** -activation. Compensatory mutational analysis revealed that non-native long-range base-pairings between the loop region of P2.1 RNA and L5c region of the **mutant** **ribozyme** are needed for the activation in spite of the fact that P2.1 forms base-pairings with P9.1 in the Tetrahymena **ribozyme**. The **trans** -activation depends on the non-native RNA-RNA interaction together with the higher order structure of P2.1 RNA. This activation is unique among the known **trans**-activations that utilize native tertiary interactions or RNA chaperons.

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09895367 99178851

Optimizing the substrate specificity of a group I **intron** **ribozyme**.

Zarrinkar PP; Sullenger BA
Center for Genetic and Cellular Therapies, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, USA.

Biochemistry (UNITED STATES) Mar 16 1999, 38 (11) p3426-32, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: GM 53525, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Group I **ribozymes** can repair **mutant** RNAs via **trans** -splicing. Unfortunately, substrate specificity is quite low for the **trans**-splicing reaction catalyzed by the group I **ribozyme** from Tetrahymenathermophila. We have used a systematic approach based on biochemical knowledge of the function of the Tetrahymena **ribozyme** to optimize its ability to discriminate against nonspecific substrates in vitro. **Ribozyme** derivatives that combine a mutation which indirectly slows down the rate of the chemical cleavage step by weakening guanosine binding with additional mutations that weaken substrate binding have greatly enhanced specificity with short oligonucleotide substrates and an mRNA fragment derived from the p53 gene. Moreover, compared to the wild-type **ribozyme**, reaction of a more specific **ribozyme** with targeted substrates is much less sensitive to the presence of nonspecific RNA competitors. These results demonstrate how a detailed understanding of the biochemistry of a catalytic RNA can facilitate the design of customized **ribozymes** with improved properties for therapeutic applications.

3/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09879178 99126527
Trans-splicing **ribozymes** for targeted gene delivery.

Kohler U; Ayre BG; Goodman HM; Haseloff J
MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England.

J Mol Biol (ENGLAND) Feb 5 1999, 285 (5) p1935-50, ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Ribozymes are potential tools for genetic manipulation, and various naturally occurring catalytic RNAs have been dissected and used as the basis for the design of new endoribonuclease activities. While such cleaving **ribozymes** may work well in vitro, they have not proved to be routinely effective in depleting living cells of the chosen target RNA. Recently, **trans**-splicing **ribozymes** have been employed to repair **mutant** mRNAs in vivo. We have designed modified **trans**-splicing **ribozymes** with improved biological activity. These allow accurate splicing of a new 3' exon sequence into a chosen site within a target RNA, and in frame fusion of the exon can result in expression of a new gene product. These **trans**-splicing **ribozymes** contain catalytic sequences derived from a self-splicing group I **intron**, which have been adapted to a chosen target mRNA by fusion of a region of extended complementarity to the target RNA and precise alteration of the guide sequences required for substrate recognition. Both modifications are required for improved biological activity of the **ribozymes**. Whereas cleaving **ribozymes** must efficiently deplete a chosen mRNA species to be effective in vivo, even inefficient **trans**-splicing can allow the useful expression of a new gene activity, dependent on the presence of a chosen RNA. We have targeted **trans**-splicing **ribozymes** against mRNAs of chloramphenicol acetyltransferase, human immunodeficiency virus, and cucumber mosaic virus, and demonstrated **trans** -splicing and delivery of a marker gene in Escherichia coli cells. The improved **trans**-splicing **ribozymes** may be tailored for virtually any

target RNA, and provide a new tool for triggering gene expression in specific cell ty[illegible]. Copyright 1999 Academic Pres[illegible]

3/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09821097 99119236
Probing the interplay between the two steps of group I **intron** splicing: competition of exogenous guanosine with omega G.
Zarrinkar PP; Sullenger BA
Center for Genetic and Cellular Therapies, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710, USA.
Biochemistry (UNITED STATES) Dec 22 1998, 37 (51) p18056-63, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: GM 53525, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
One largely unexplored question about group I **intron** splicing is how the cleavage and ligation steps of the reaction are coordinated. We describe a simple in vitro **trans**-splicing model system in which both steps take place, including the exchange of ligands in the guanosine-binding site that must occur between the two steps. Using this model system, we show that the switch is accomplished by modulating the relative affinity of the binding site for the two ligands. While the terminal guanosine of the **intron** (omegaG) and exogenous guanosine compete for binding during the first step of splicing, no competition is apparent during the second step, when omegaG is bound tightly. These results help explain how the **ribozyme** orchestrates progression through the splicing reaction. In addition to providing a new tool to ask basic questions about RNA catalysis, the **trans**-splicing model system will also facilitate the development of therapeutically useful group I **ribozymes** that can repair **mutant** mRNAs.

3/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09706476 98407947
Ribozymes as therapeutic tools for genetic disease.
Phylactou LA; Kilpatrick MW; Wood MJ
Department of Human Anatomy, Oxford University, South Parks Road, Oxford OX1 3QX, UK. leonidas.phylactou@anat.ox.ac.uk
Hum Mol Genet (ENGLAND) 1998, 7 (10) p1649-53, ISSN 0964-6906 Journal Code: BRC
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
The discovery that RNA can act as a biological catalyst, as well as a genetic molecule, indicated that there was a time when biological reactions were catalysed in the absence of protein-based enzymes. It also provided the platform to develop those catalytic RNA molecules, called **ribozymes**, as **trans** -acting tools for RNA manipulation. Viral diseases or diseases due to genetic lesions could be targeted therapeutically through **ribozymes**, provided that the sequence of the genetic information involved in the disease is known. The hammerhead **ribozyme**, one of the smallest **ribozymes** identified, is able to induce site-specific cleavage of RNA, with **ribozyme** and substrate being two different oligoribonucleotides with regions of complementarity. Its ability to down-regulate gene expression through RNA cleavage makes the hammerhead **ribozyme** a candidate for genetic therapy. This could be particularly useful for dominant genetic diseases by down-regulating the expression of **mutant** alleles. The group I **intron ribozyme**, on the other hand, is capable of site-specific RNA **trans** -splicing.

It can be engineered to replace part of an RNA with sequence attached to its 3' end. Such application may have importance in the repair of **mutant** mRNA molecules giving rise to genetic diseases. However, to achieve successful **ribozyme** -mediated RNA-directed therapy, several parameters including **ribozyme** stability, activity and efficient delivery must be considered. **Ribozymes** are promising genetic therapy agents and should, in the future, play an important role in designing strategies for the therapy of genetic diseases.

3/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09663279 98409449
Identifying RNA minor groove tertiary contacts by nucleotide analogue interference mapping with N2-methylguanosine.
Ortoleva-Donnelly L; Kronman M; Strobel SA
Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA.
Biochemistry (UNITED STATES) Sep 15 1998, 37 (37) p12933-42, ISSN 0006-2960 Journal Code: A0G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Nucleotide analogue interference mapping (NAIM) is a general biochemical method that rapidly identifies the chemical groups important for RNA function. In principle, NAIM can be extended to any nucleotide that can be incorporated into an in vitro **transcript** by an RNA polymerase. Here we report the synthesis of 5'-O-(1-thio)-N2-methylguanosine triphosphate (m2GalphaS) and its incorporation into two reverse splicing forms of the Tetrahymena group I **intron** using a **mutant** form of T7 RNA polymerase. This analogue replaces one proton of the N2 exocyclic amine with a methyl group, but is as stable as guanosine (G) for secondary structure formation. We have identified three sites of m2GalphaS interference within the Tetrahymena **intron**: G22, G212, and G303. All three of these guanosine residues are known to utilize their exocyclic amino groups to participate in tertiary hydrogen bonds within the **ribozyme** structure. Unlike the interference pattern with the phosphorothioate of inosine (IalphaS, an analogue that deletes the N2 amine of G), m2GalphaS substitution did not cause interference at positions attributable to secondary structural stability effects. Given that the RNA minor groove is likely to be widely used for helix packing, m2GalphaS provides an especially valuable reagent to identify RNA minor groove tertiary contacts in less well-characterized RNAs.

3/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09504560 98207035
Trans-activation of the Tetrahymena **ribozyme** by its P2-2.1 domains.
Ikawa Y; Shiraishi H; Inoue T
Department of Chemistry, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.
J Biochem (Tokyo) (JAPAN) Mar 1998, 123 (3) p528-33, ISSN 0021-924X Journal Code: HIF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The Tetrahymena group I self-splicing **intron** contains peripheral domains P2-2.1. **Mutant introns** lacking these domains are hardly active. We found that if an independently prepared P2-2.1 RNA is added in **trans**, it efficiently enhances the catalytic activity of an **intron** lacking the domains. P2-2.1 RNA together with the previously

identified activator, P5abc RNA, of the Tetrahymena **intron** can activate the **intron** lacking both of them. The **trans**-activation depends on the long-range interaction between P2.1 and P9.1 domains.

3/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09455066 98191345

Sequence specificity of a group II **intron ribozyme**: multiple mechanisms for promoting unusually high discrimination against mismatched targets.

Xiang Q; Qin PZ; Michels WJ; Freeland K; Pyle AM
Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York 10032, USA.

Biochemistry (UNITED STATES) Mar 17 1998, 37 (11) p3839-49, ISSN 0006-2960 Journal Code: A0G
Contract/Grant No.: GMR0150313
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Group II **intron** ai5 gamma was reconstructed into a multiple-turnover **ribozyme** that efficiently cleaves small oligonucleotide substrates in-**trans**. This construct makes it possible to investigate sequence specificity, since second-order rate constants (kcat/K(m), or the specificity constant) can be obtained and compared with values for **mutant** substrates and with other **ribozymes**. The **ribozyme** used in this study consists of **intron** domains 1 and 3 connected in-cis, together with domain 5 as a separate catalytic cofactor. This **ribozyme** has mechanistic features similar to the first step of reverse-splicing, in which a lariat **intron** attacks exogenous RNA and DNA substrates, and it therefore serves as a model for the sequence specificity of group II **intron** mobility. To quantitatively evaluate the sequence specificity of this **ribozyme**, the WT kcat/Km value was compared to individual kcat/Km values for a series of **mutant** substrates and **ribozymes** containing single base changes, which were designed to create mismatches at varying positions along the two **ribozyme** -substrate recognition helices. These mismatches had remarkably large effects on the discrimination index (1/relative kcat/K(m)), resulting in values > 10,000 in several cases. The delta delta G++ for mismatches ranged from 2 to 6 kcal/mol depending on the mismatch and its position. The high specificity of the **ribozyme** is attributable to effects on duplex stabilization (1-3 kcal/mol) and unexpectedly large effects on the chemical step of reaction (0.5-2.5 kcal/mol). In addition, substrate association is accompanied by an energetic penalty that lowers the overall binding energy between **ribozyme** and substrate, thereby causing the off-rate to be faster than the rate of catalysis and resulting in high specificity for the cleavage of long target sequences (> or = 13 nucleotides).

3/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09051256 97271991

Suppressors of cis-acting splicing-deficient mutations that affect the **ribozyme** core of a group II **intron**.

Robineau S; Bergantino E; Carignani G; Michel F; Netter P
Centre de Genetique Moleculaire du CNRS (associe a l'Universite Pierre et Marie CURIE), Gif-sur-Yvette, France.

J Mol Biol (ENGLAND) Apr 4 1997, 267 (3) p537-47, ISSN 0022-2836
Journal Code: J6V
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Many of the cis-dominant mutations that lead to respiratory deficiency by preventing maturation of specific yeast mitochondrial **transcripts** are found to affect the **ribozyme** core of group I and group II **introns**. We have searched for suppressors of mutations in the **ribozyme**-encoding sections of a group II **intron**, the first **intron** in the COX1 gene of Saccharomyces cerevisiae, which was independently subjected to in vitro site-directed mutagenesis. Three of the original **mutants** bore multiple mutations, which act synergistically, since for most individual mutations, suppressors could be obtained that ensured at least partial recovery of respiratory competence and splicing. Out of a total of ten suppressor mutations that were identified, three were second-site substitutions that restored postulated base-pairings in the **ribozyme** core. Remarkably, and as is observed for group I **introns**, at least half of the cis-dominant mutations in the first two group II **introns** of the COX1 gene affect sites that have been shown to participate in RNA tertiary interactions. We propose that this bias reflects cooperativity in the formation of **ribozyme** tertiary but not secondary structure, on the one hand, and the need for synergistic effects in order to generate a respiratory-deficient phenotype in the laboratory on the other. Finally, a novel in vivo splicing product of **mutant** cells is attributed to bimolecular splicing at high concentrations of defective **transcripts**.

3/3,AB/10 (Item 10 from file: 155)

09029548 97250359

Branch-site selection in a group II **intron** mediated by active recognition of the adenine amino group and steric exclusion of non-adenine functionalities.

Liu Q; Green JB; Khodadadi A; Haeberli P; Beigelman L; Pyle AM
Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032, USA.
J Mol Biol (ENGLAND) Mar 21 1997, 267 (1) p163-71, ISSN 0022-2836
Journal Code: J6V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The 2'-hydroxyl on a specific bulged adenosine is the nucleophile during the first step of splicing by group II **introns**. To understand the means by which the **ribozyme** core recognizes this adenosine, it was mutagenized and effects on catalytic activity were quantified. The results indicate that a low level of mutational variability is tolerated at the branch-site of group II **introns**, with no apparent loss of fidelity. Analyses of **mutant** and modified nucleotides at the branch-site reveal that adenine is recognized primarily through the N6 amino group and by steric exclusion of functionalities found on other bases. The mutational and single atom effects reported here contrast with those observed during spliceosomal processing, suggesting that there are important differences in adenosine recognition by the two systems.

3/3,AB/11 (Item 11 from file: 155)

08628375 96069407

Cotranscriptional splicing of a group I **intron** is facilitated by the Cbp2 protein.

Lewin AS; Thomas J Jr; Tirupati HK
Department of Molecular Genetics and Microbiology, University of Florida College of Medicine, Gainesville 32610-0266, USA.
Mol Cell Biol (UNITED STATES) Dec 1995, 15 (12) p6971-8, ISSN 0270-7306 Journal Code: NGY

Contract/Grant No.: GM12228, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The nuclear CBP2 gene encodes a protein essential for the splicing of a mitochondrial group I **intron** in Saccharomyces cerevisiae. This **intron** (bI5) is spliced autocatalytically in the presence of high concentrations of magnesium and monovalent salt but requires the Cbp2 protein for splicing under physiological conditions. Addition of Cbp2 during RNA synthesis permitted cotranscriptional splicing. Splicing did not occur in the **transcription** buffer in the absence of synthesis. The Cbp2 protein appeared to modify the folding of the **intron** during RNA synthesis: pause sites for RNA polymerase were altered in the presence of the protein, and some **mutant transcripts** that did not splice after **transcription** did so during **transcription** in the presence of Cbp2. Cotranscriptional splicing also reduced hydrolysis at the 3' splice junction. These results suggest that Cbp2 modulates the sequential folding of the **ribozyme** during its synthesis. In addition, splicing during **transcription** led to an increase in RNA synthesis with both T7 RNA polymerase and mitochondrial RNA polymerase, implying a functional coupling between **transcription** and splicing.

3/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08498677 96051282
Suppression of lung cancer cell growth by **ribozyme**-mediated modification of p53 pre-mRNA.

Cai DW; Mukhopadhyay T; Roth JA
Department of Thoracic and Cardiovascular Surgery, University of Texas M.D. Anderson Cancer Center, Houston 77030, USA.

Cancer Gene Ther (UNITED STATES) Sep 1995, 2 (3) p199-205, ISSN 0929-1903 Journal Code: CE3
Contract/Grant No.: NCI 16672; RO1 CA 45187, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

An anti-p53 **ribozyme** (catalytic RNA) designed to cleave the p53 pre-messenger RNA (mRNA) can efficiently reduce the level of endogenous **mutant** p53 mRNA. Retrovirus-mediated **transduction** of a hammerhead **ribozyme** (Rz5a) designed to cleave unspliced p53 RNA at codon 187 near the boundary of **intron** 5 and exon 6 reduced the level of **mutant** p53 RNA and protein in the human H226Br lung cancer cell line, which contains a homozygous p53 mutation at codon 254. The catalytic cleavage of the p53 pre-mRNA but not the p53 mRNA by the **ribozyme** was shown in vitro. The cleavage of the p53 pre-mRNA by this **ribozyme** was specific because a mutation in its catalytic domain (Rz5m) abolished the cleavage activity in vitro. Expression of the Rz5a **ribozyme** significantly suppressed the growth of the H226Br cells in culture. However, another **ribozyme** (Rz7a) targeted at codon 264 of the p53 gene near the boundary of **intron** 7 and exon 8 showed in vitro cleavage of the pre-mRNA but did not suppress cell growth. The site of modification in the p53 pre-mRNA may determine the degree of **ribozyme** -mediated growth suppression in this cell line. Our findings that p53 pre-mRNA can be modified by a specific **ribozyme** in vivo suggest a possible role for these agents in gene therapy strategies for cancer.

3/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07984527 94350967
Tetrahymena **ribozyme** disrupts rRNA processing in yeast.

Good L; Elela SA; Nazar RN

Department of Molecular Biology and Genetics, University of Guelph, Ontario, Canada.
J Biol Chem (UNITED STATES) Sep 2 1994, 269 (35) p22169-72, ISSN 0021-9258 Journal Code: HIV
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The intervening sequence (IVS) of Tetrahymena thermophila nucleolar DNA interrupts a highly conserved sequence in the RNA core structure of the large ribosomal subunit. This location in nuclear DNA is unusual as most group I **introns** are in mitochondrial and chloroplast DNA. To examine the effect of a **ribozyme** insertion in another nuclear genome, the Tetrahymena IVS was introduced into the analogous position in a cloned Schizosaccharomyces pombe ribosomal gene, and the **mutant** rDNA was expressed in vivo. RNA analyses indicated that mature 5.8 S rRNA was not formed from the **mutant** gene **transcript** and the amount of 27 S nRNA was significantly reduced. In contrast, hybridization analyses indicated that RNA splicing continued, and normal forms of free **ribozyme** were present. The results show that the IVS sequence can interfere with rRNA processing and suggest that the unusual amplification of a single rDNA repeat may have forced Tetrahymena to accommodate its **ribozyme**.

3/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07939253 94283392
Two major tertiary folding transitions of the Tetrahymena catalytic RNA.
Laggerbauer B; Murphy FL; Cech TR
Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado, Boulder 80309-0215.
EMBO J (ENGLAND) Jun 1 1994, 13 (11) p2669-76, ISSN 0261-4189
Journal Code: EMB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The L-21 Tetrahymena **ribozyme** , an RNA molecule with sequence-specific endoribonuclease activity derived from a self-splicing group I **intron**, provides a model system for studying the RNA folding problem. A 160 nucleotide, independently folding domain of tertiary structure (the P4-P6 domain) comprises about half of the **ribozyme**. We now apply Fe(II)-EDTA cleavage to **mutants** of the **ribozyme** to explore the role of individual structural elements in tertiary folding of the RNA at equilibrium. Deletion of peripheral elements near the 3' end of the **ribozyme** destabilizes a region of the catalytic core (P3-P7) without altering the folding of the P4-P6 domain. Three different mutations within the P4-P6 domain that destabilize its folding also shift the folding of the P3-P7 region of the catalytic core to higher $MgCl_2$ concentrations. We conclude that the role of the extended P4-P6 domain and of the 3'-terminal peripheral elements is at least in part to stabilize the catalytic core. The organization of RNA into independently folding domains of tertiary structure may be common in large RNAs, including ribosomal RNAs. Furthermore, the observation of domain-domain interactions in a catalytic RNA supports the feasibility of a primitive spliceosome without any proteins.

3/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07637717 93280161
Mutation of the conserved first nucleotide of a group II **intron** from yeast mitochondrial DNA reduces the rate but allows accurate splicing.
Peebles CL; Belcher SM; Zhang M; Dietrich RC; Perlman PS

Department of Biological Sciences, University of Pittsburgh, Pennsylvania 15260.
J Biol Chem (UNITED STATES) Jun 5 1993, 268 (16) p11929-38, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: GM31480, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Group II **introns** have a phylogenetically conserved 5'-terminal pentanucleotide, -G1U2G3C4G5-, that resembles the conserved 5' end sequence of nuclear pre-mRNA **introns**. No functional interaction or catalytic role for the conserved G1 position has been proposed, although a tertiary structure involving -G3C4- has been implicated in splicing in vitro. We have analyzed splicing phenotypes both in vitro and in vivo for all three point **mutants** affecting guanosine at position 1 (G1) of **intron** 5 gamma from the COXI gene of yeast mitochondrial DNA. While all of these G1N substitutions slow splicing in vitro, G1C is clearly the most defective. All three **mutant transcripts** splice as accurately as the wild-type **transcript**, although the yield of lariat **intron** is reduced. The branched trinucleotide core includes the mutated position 1 nucleotide linked to the canonical branchpoint adenosine. The **mutant** lariats vary significantly in their susceptibility to the debranching activity from human cells. After wild-type, G1A was most sensitive, G1U was somewhat resistant, while G1C was highly resistant to debranching. These **mutant** lariats had normal **ribozyme** activity for promoting spliced exon reopening. The three **mutant introns** were transformed into otherwise normal yeast mitochondrial DNA. These **mutants** grow on nonfermentable carbon sources and splce aI5 gamma to yield excised **intron** lariat and mRNA. Nonetheless, each **mutant** splices with reduced efficiency, roughly parallel to their in vitro activity. In vivo, all three **mutants** accumulate both the pre-mRNA retaining **intron** 5 gamma and the lariat splicing intermediate containing **intron** and 3' exon. Clearly, this primary sequence element, shared with nuclear pre-mRNA **introns**, has a very different functional significance in group II splicing.

3/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06996151 91269319
Splice site selection and role of the lariat in a group II **intron**.
Jacquier A; Jacquesson-Breuleux N
Unite de genetique moleculaire des levures (URA 1149 du CNRS), Institut Pasteur, Paris, France.
J Mol Biol (ENGLAND) Jun 5 1991, 219 (3) p415-28, ISSN 0022-2836 Journal Code: J6V
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The structural elements involved in 5' and 3' splice site (SS) selection in a group II **intron** were analyzed. While 5' SS selection appears to be defined by only one element, the EBS1-IBS1 pairing, four distinct structural components contribute to 3' SS selection, one of which being analogous to the "internal guide sequence" described for group I **introns**. Moreover, some of the **mutants** analyzed during this study induce efficient 5' SS hydrolysis and suggest how 5' SS transesterification is selected against hydrolysis. Finally, the lariat structure was found to accelerate both steps of splicing, suggesting that it "locks" the **ribozyme** in an active configuration.

3/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06690319 91182746

Effects of mutations of the bulged nucleotide in the conserved P7 pairing element of the phage T4 td **intron** on **ribozyme** function.

Schroeder R; von Ahsen U; Belfort M

Wadworth Center for Laboratories and Research, New York State Department of Health, Albany 12201.

Biochemistry (UNITED STATES) Apr 2 1991, 30 (13) p3295-303, ISSN 0006-2960 Journal Code: A0G

Contract/Grant No.: GM39422, GM, NIGMS; GM44844, GM, NIGMS

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The P7 element of group I **introns** contains a semiconserved "bulged" nucleotide, a C in group IA **introns** (nt 870 in the td **intron**) and an A in group IB **introns** [Cech, T.R. (1988) Gene 73, 259-271]. Variants U870, G870, and A870, isolated by a combination of in vitro and in vivo genetic strategies, indicate that C and A at position 870 are consistent with splicing whereas U and G are not. Although **mutants** G870 and U870 could be activated in vitro by increasing the Mg^{2+} concentration, their Km for GTP at pH 7 was 20-100-fold elevated, and they were unable to undergo site-specific hydrolysis. The dependence of the **mutants** on high guanosine concentrations could be substantially overcome by an increase in pH, suggesting that a tautomeric change, which makes U and G mimic C and A, is responsible for restoring function. In contrast to the striking Km effect, Vmax for the **mutants** differed by less than a factor of 2 from the wild type. Furthermore, streptomycin, an aminoglycoside antibiotic that competes with guanosine for its binding site, inhibited splicing of the U870 and G870 constructs at least as well as of the C870 and A870 variants, indicating that the guanosine-binding site of the **mutants** is proficient at interacting with a guanidino group. While our experiments argue against a hydrogen-bonding interaction between the C6-O of the cofactor and C4-NH2 of the bulged nucleotide, they are consistent with other models in which the C4-NH2 and/or N3 groups of the bulged C are involved in establishing an active **ribozyme**.

3/3,AB/18 (Item 1 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

12201815 BIOSIS NO.: 199900496664

Evaluating group I **intron** catalytic efficiency in mammalian cells.

AUTHOR: Long Meredith B; Sullenger Bruce A(a)

AUTHOR ADDRESS: (a)DUMC, Durham, NC, 27710**USA

JOURNAL: Molecular and Cellular Biology 19 (10):p6479-6487 Oct., 1999

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DOCUMENT TYPE: Article

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LANGUAGE: English

SUMMARY LANGUAGE: English

ABSTRACT: Recent reports have demonstrated that the group I **ribozyme** from Tetrahymena thermophila can perform **trans**-splicing reactions to repair **mutant** RNAs. For therapeutic use, such **ribozymes** must function efficiently when **transcribed** from genes delivered to human cells, yet it is unclear how group I splicing reactions are influenced by intracellular expression of the **ribozyme**. Here we evaluate the self-splicing efficiency of group I **introns** from **transcripts** expressed by RNA polymerase II in human cells to directly measure **ribozyme** catalysis in a therapeutically relevant setting. **Intron**-containing expression cassettes were transfected into a human cell line, and RNA **transcripts** were analyzed for **intron** removal. The percentage of **transcripts** that underwent self-splicing ranged from 0 to 50%, depending on the construct being tested. Thus, self-splicing activity is supported in the mammalian cellular environment. However, we find that the extent of self-splicing

is greatly influenced by sequences flanking the **intron** and presumably reflects differences in the **intron's** ability to fold into an active conformation inside the cell. In support of this hypothesis, we show that the ability of the **intron** to fold and self-splice from cellular **transcripts** in vitro correlates well with the catalytic efficiency observed from the same **transcripts** expressed inside cells. These results underscore the importance of evaluating the impact of sequence context on the activity of therapeutic group I **ribozymes**. The self-splicing system that we describe should facilitate these efforts as well as aid in efforts at enhancing in vivo **ribozyme** activity for various applications of RNA repair.

3/3,AB/19   (Item 2 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)

12188296   BIOSIS NO.: 199900483145
Evolutionary analyses of small-subunit rDNA coding regions and the 1506 group I **introns** of the Zygnematales (Charophyceae, Streptophyta).
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JOURNAL: Journal of Phycology 35 (3):p560-569 June, 1999
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RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: We sequenced the small-subunit (SSU) rDNA coding region and 1506 group I **intron** (that interrupts this gene) in Closterium ehrenbergii Menegh. ex Ralfs, Closterium littorale Gay, Cylindrocystis brebissonii (Menegh.) de Bary, Penium margaritaceum (Ehr.) de Breb. ex Ralfs, and Staurastrum punctulatum de Breb. and the 1506 **intron** in Sirogonium sticticum (J.E. Smith) Kutz. (Zygnematales). Reverse **transcriptase** (RT)/PCR analyses demonstrated that the 1506 **introns** are not present in vivo in mature rRNA. Splicing analyses in vitro showed, however, that these **introns** do not catalyze their own excision from the rRNA coding region. Mutation of the conserved G (omegaG) to an A at the 3' terminus of the 1506 **intron** of Gonatozygon aculeatum Hastings (Zygnematales) restored self-splicing activity. This **mutant intron** utilized a new 3' splice site to facilitate autoexcision. We speculate that the 3' terminus of the wild-type 1506 **intron** may be a target for a "helper" factor that facilitates the second step of splicing (exon ligation) in vivo in the Zygnematales. Phylogenetic analyses showed congruence of rDNA and **intron** trees confirming an ancient origin of the 1506 **intron** in the common ancestor of the Zygnematales. The rDNA trees were compared to those inferred from rbcL sequence analyses. These trees were in general agreement and showed polyphyly of the Mesotaeniaceae and the Zygnemataceae. The 1506 **introns** contain significant evolutionary signal and provided strong phylogenetic support for groups within the Zygnematales when analyzed alone or in combination with the SSU rDNA coding regions. The secondary structure of 1506 group I **introns** was studied to identify RNA elements that may be useful as systematic markers. This analysis showed an optional helix found in RNA domain P2 that was lost in a monophyletic group of Desmidiaceae. This helix is found in all other 1506 group I **introns**, including those interrupting red algal and fungal SSU rDNAs.

3/3,AB/20   (Item 3 from file: 5)
DIALOG(R)File   5:Biosis Previews(R)

08876563 BIOSIS NO.: 199396028064
An independently folding domain of RNA tertiary structure within the Tetrahymena **ribozyme**.
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JOURNAL: Biochemistry 32 (20):p5291-5300 1993
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Tetrahymena thermophila pre-rRNA contains a 413-nucleotide self-splicing group I **intron**. This **intron** has been converted into a sequence-specific endonuclease or **ribozyme**. A 160-nucleotide portion of the **ribozyme** consisting of both highly conserved sequence elements (P4 and P6) and nonconserved peripheral extensions (P5abc and P6ab) was synthesized as a separate molecule. Solvent-based Fe(II)EDTA, a probe that monitors higher-order RNA structure, revealed a protection pattern that was a large subset of that observed in the whole **ribozyme**. Data from dimethyl sulfate modification and partial digestion with nucleases were also consistent with maintenance of the proper secondary and tertiary structure in the shortened RNA molecule. Thus, this 160-nucleotide molecule (P4-P6 RNA) is an independently folding domain of RNA tertiary structure. A series of mutations and deletions were made within the P4-P6 domain to further dissect its tertiary structure. Fe(II)-EDTA and dimethyl sulfate analysis of these **mutants** revealed that the domain consists of two substructures, a localized subdomain involving the characteristic adenosine-rich bulge in P5a, and a subdomain-stabilized structure involving long-range interactions. Therefore, like some proteins, the **intron** RNA is modular, containing a separable domain and subdomain of tertiary structure.

3/3,AB/21 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08066320 BIOSIS NO.: 000093087768
THE P2 ELEMENT OF THE TD **INTRON** IS DISPENSABLE DESPITE ITS NORMAL ROLE IN SPLICING
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JOURNAL: J BIOL CHEM 267 (5). 1992. 2845-2848.
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CODEN: JBCHA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The P2 region of group I **introns** has been proposed to be involved in the correct positioning of the P1 5'-splice site duplex in the catalytic core (Michel, F., and Westhof, E. (1990) J. Mol. Biol. 216, 585-610). The behavior of .DELTA.P2 deletion **mutants** of the td **intron** is consistent with this hypothesis. The .DELTA.P2 **mutants** are capable of site-specific hydrolysis, indicating that the conformation of the **ribozyme** is not grossly altered, but they are incapable of **transesterification** reactions at the splice sites, as would be predicted if P1 is not appropriately aligned within the catalytic core. Nevertheless, the function of the P2 element can be bypassed in specific pseudorevertants isolated by genetic selection from the .DELTA.P2 **mutants**. These results, together with phylogenetic data, support the existence of alternate strategies to create a